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Sandra Saucier
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for 10/054419
1
AN 134:233815 CA
TI Physical perturbation for fluorescent characteri: ation of microorganism particles
AU Bronk, Burt V.; Shoaibi, Azadeh; Nudelman, R aphael; Akinyemi, Agnes N.
SO Proceedings of SPIE-The International Societ for Optical Engineering (2000), 4036(Chemical and Biological Sensing), 169-180.

2.
AN 131:141627 CA
TI Fluorescence of dipicolinic acid as a possible component of the observed UV emission spectra of bacterial spores
AU Nudelman, Raphael; Feay, Nicole; Hirsch, Mat iew; Efrima, Shlomo; Bronk, Burt
SO Proceedings of SPIE-The International Society for Optical Engineering (1999), 3533(Air Monitoring and Detection of Ch mical and Biological Agents), 190-195

3
AN 135:192324 CA
TI Ultraviolet fluorescence imaging applications
AU Hill, Ralph H., Jr.; Angell, Peter
SO AT-PROCESS (2000), 5(3,4), 108-114

4.
AN 123:51436 CA
TI Spectroscopic properties of tryptophan and bact ria
AU Tang, G. C.; Yang, Y. L.; Huang, Z. Z.; Hua, W Zhou, F.; Cosloy, S.; Alfano, R. R.
SO Proceedings of SPIE-The International Society or Optical Engineering (1995), 2387, 169-72

5.
AN 120:265052 CA
TI Online, non-destructive biomass determination o bacterial biofilms by fluorometry
AU Angell, Peter; Arrage, Andrew A.; Mittelman, M rc W.; White, David C.
SO Journal of Microbiological Methods (1993), 18(4), 317-27

Spectroscopic Propeties of Tryptophan and Bacteria

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I. ABSTRACT

Fluorescence spectra of tryptophan and bacteria were measured at different concentrations using a Mediscience CD-Scan unit. The emission spectra of tryptophan were obtained using an excitation wavelength at 280 nm. The excitation spectra were obtained at the emission of 340 nm. The minimum detectable concentration of tryptophan was found to be 10^{-8} M. The emission spectra for bacteria were probed at 340 nm. The minimum detectable number of bacteria in a beam of the excitation light was determined to be about 30. Assuming that the emission band at 340 nm of bacteria comes from tryptophan, the number of tryptophan per bacterium was estimated to be 10^{8}. This approach to determine the number is almost consistent with that obtained using a weight method.

II. INTRODUCTION

Over the years, fluorescence spectroscopy have been used as a means to study bacteria by several groups[1]. The motivation of these efforts is to find a sensitive and nondestructive method to detect the presence of bacteria and biological organisms.

Most bacteria emit intense fluorescence primarily from proteins and flavins when excited by ultraviolet or visible light. Proteins show intense fluorescence emission peaked at 340 nm when they are excited by 280 nm UV light. The intense fluorescence from a complex protein structure originates from the aromatic amino acid residues such as tryptophan, tyrosine and phenylalanine. The fluorescence is primarily attributed to tryptophan residues because the fluorescence from phenylalanine is small and is rarely more than 10% from tyrosine when one or more tryptophan residues are present in a protein. Tryptophan is a excellent marker to determine the presence of bacteria.

In this report, we describe the fluorescence intensity of tryptophan and bacteria at different concentrations. A method is described to calculate the number of tryptophan molecules in a bacterium.

III. MATERIAL and EXPERIMENTAL

The bacterium Escherichia coli (PWH542) was used in our study. It was obtained from the American Type Culture Collection, Rockville, Maryland. Stock cultures were maintained on penassay broth slants at 4^{0}C, following incubation at the proper growth in luria-bertani medium at 37^{0}C for 24 hours. Bacteria were washed three times with 5 ml PBS, and then they were pelleted by centrifugation of 5000 x g for 5 min. at 4^{0}C. These bacteria were suspended in phosphate buffer, pH 7.0, prior to spectral measurements. Bacterial suspensions were stored at 4^{0}C and were allowed to equilibrate to room temperature prior spectral study. DL-tryptophan was obtained from Gibco Laboratory, New York. Distilled water was used to prepare all tryptophan solutions.

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The fluorescence emission and excitation spectra were measured with Mediscience Technology Corp. CD-Scanner based on Perkin-Elmer LS-50 luminescence spectrometer. CD-Scanner can be used to obtain both emission and excitation spectral profiles by fixing a excitation or emission wavelength and scanning the other wavelengths. A quartz cell of 1x1x3.5 cm3 was used for all spectral measurements

IV. RESULTS

Fluorescence emission spectra of tryptophan and bacteria were measured using a volume excitation method at an excitation wavelength of 280 nm for different concentrations. Fluorescence spectral profiles were measured from 300 to 540 nm for all of samples. Fluorescence excitation spectra from 200 to 320 nm were measured at the emission peak of 340 nm.

Fig.1 shows a typical fluorescence emission spectrum of DL-tryptophan at 10^{-4} M. The spectral peak is located at 358 nm. Fig.2 shows fluorescence emission intensity at 340 nm versus tryptophan concentration. Over 10^{-8} to 10^{-4} M, the curve is linear.

Fig.3 shows a fluorescence emission spectrum of bacteria ATCC542 at a density of $7.02x10^5$/ml. The spectral peak is located at 345 nm. Fig.4 shows the relationship between the fluorescence emission intensity at 340 nm and bacterial density. The fluorescence intensity vs density over the density range of $7.02x10^4$ to 10^8/ ml is linear.

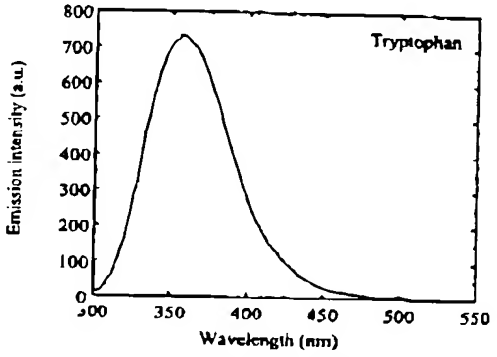

Fig.1 Fluorescence emission spectrum of tryptophan solution excited at 280 nm

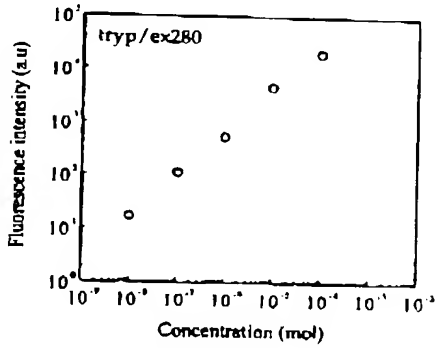

Fig.2. Fluorescence emission intensity of trytophan solution vs concentrantion

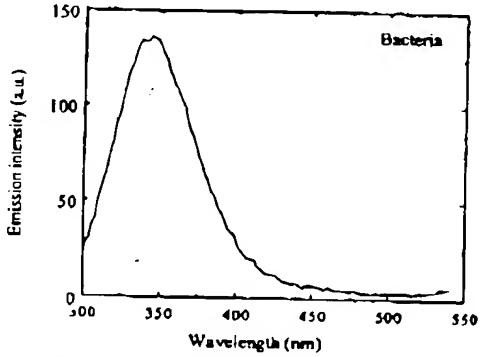

Fig.3 Fluorescence emission spectrum of bacteria excited at 280 nm

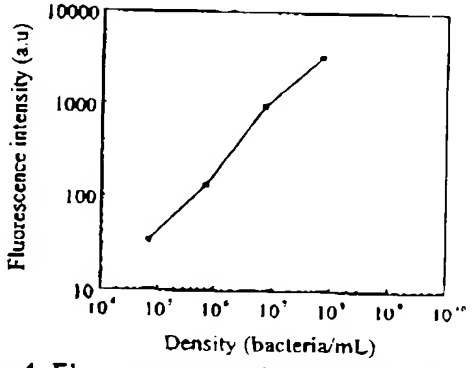

Fig.4 Fluorescence emission intensity of bacteria vs intensity

A typical fluorescence excitation spectrum of DL-tryptophan of 10^{-4} M is shown in Fig.5. There are two peaks in the excitation spectral profile. One is located at 224 nm, and other at 278 nm. Fig.6 shows the relationship between the fluorescence excitation intensity at 280 nm and tryptophan concentration. The excitation intensity vs concentration over the concentration range of 10^{-8} to 10^{-4} M is linear.

The fluorescence excitation spectrum of bacteria ATCC542 is shown in Fig.7 at a density of 7.02×10^5. There are two peaks in the excitation spectral profile. One is located at 225 nm and the other at about 280 nm . Fig.8 shows the relationship between the fluorescence excitation intensity at 280 nm and bacterium density. The fluorescence excitation intensity vs bacterium density over the range of 7.02×10^4 to 10^8/ml is linear.

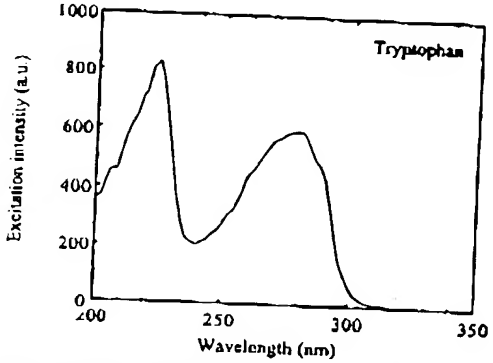

Fig.5 Fluorescence excitation spectrum of tryptophan solution emitted at 340 nm

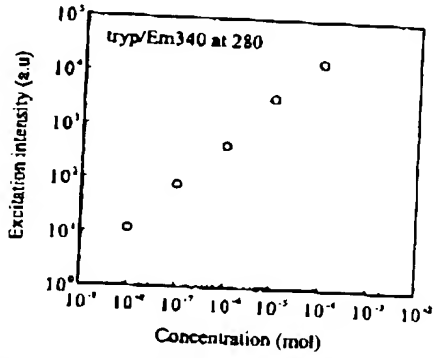

Fig.6. Fluorescence excitation intensity of trytophan solution vs concentrantion

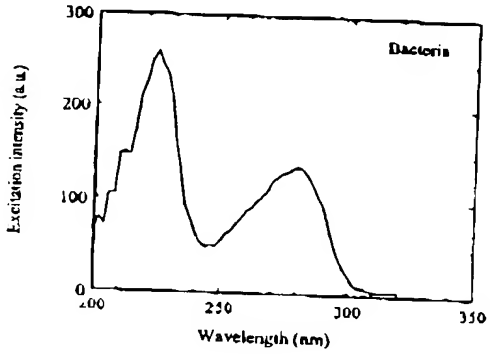

Fig.7 Fluorescence excitaion spectrum of bacteria emitted at 340 nm

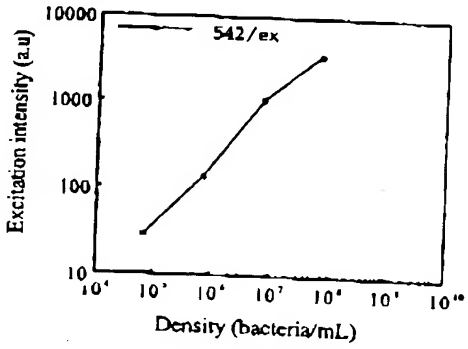

Fig.8 Fluorescence excitation intensity of bacteria vs intensity

V. DISCUSSION

The fluorescence emission and excitation spectral profiles of bacteria was found to be similar and are characteristic of tryptophan. The emission spectral peak of bacteria shifts slightly to shorter wavelengths. It appears the fluorescence from bacteria was indeed from tryptophan[2-4]. The emission peak blue-shift of bacteria as compared with pure tryptophan is attributed to environmental changes.

An important and interested result can be obtained by compare the bacterial fluorescence intensity with fluorescence intensity of tryptophan. One can estimate how many tryptophan molecules are in a bacterium. The detectable level of tryptophan molecules is about 10^{-8} M with a CD-Scanner using emission and excitation method. The detectable level of bacteria is about $7x10^4$/ml. From these data, the number of tryptophan per bacterium is estimated to be about $9x10^7$. This result is consistent with that obtained using a weight method[5] which gave $6x10^7$. The optical approach appears much simpler and faster.

ACKNOWLEDGMENTS

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